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Ontogeny of NET expression and antidepressant-like response to desipramine in wild-type and SERT mutant mice.

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ABSTRACT

Depression is a major public health concern with symptoms that are often poorly controlled by treatment with common antidepressants. This problem is compounded in juveniles and adolescents, where therapeutic options are limited to selective serotonin reuptake inhibitors (SSRIs). Moreover, therapeutic benefits of SSRIs are often especially limited in certain subpopulations of depressed patients, including children and carriers of low expressing serotonin transporter (SERT) gene variants. Tricyclic antidepressants (TCAs) offer an alternative to SSRIs; however, how age and SERT expression influence antidepressant response to TCAs is not understood. We investigated the relation between antidepressant-like response to the TCA desipramine using the tail suspension test and saturation binding of [^{3}H]nisoxetine to the norepinephrine transporter (NET), the primary target of desipramine, in juvenile (21 days post-natal [P21]), adolescent (P28) and adult (P90) wild-type (SERT+/+) mice. To model carriers of low expressing SERT gene variants, we used mice with reduced SERT expression (SERT+/-) or lacking SERT (SERT-/-). Maximal antidepressant-like effect and potency of desipramine were greater in P21 mice than in P90 mice, and were SERT genotype independent. NET expression decreased with age in the locus coeruleus and increased with age in several terminal regions (e.g., CA1 and CA3 regions of hippocampus). Binding affinity of [^{3}H]nisoxetine did not vary as a function of age or SERT genotype. These data show age-dependent shifts for desipramine to produce antidepressant-like effects that correlate with NET expression in the locus coeruleus and suggest that drugs with NET blocking activity may be an effective alternative to SSRIs in juveniles.

INTRODUCTION

Depression is a major public health concern with symptoms often poorly controlled with commonly prescribed antidepressants. This problem is compounded in juveniles and adolescents by fewer pharmacological treatment options compared to adults (Bylund and Reed, 2007). The Food and Drug Administration (FDA) has approved two antidepressant drugs, fluoxetine and escitalopram, for use in treating pediatric depression. Both drugs are selective serotonin (5-HT) reuptake inhibitors (SSRIs), which prevent 5-HT uptake via the 5-HT transporter (SERT). SSRIs can be effective treatments for adult patients suffering from depression, but often fail to relieve all depressive symptoms (Kirsch et al., 2008). The therapeutic benefit of SSRIs can be especially limited in children and in carriers of a common SERT gene variant that yields lower SERT expression (Kessler et al., 2001; Serretti et al., 2007; Bujoreanu et al., 2011). Tricyclic antidepressants (TCAs) are an alternative to SSRIs. However, in juveniles and adolescents, TCAs are not FDA approved for the treatment of depression, and are prescribed infrequently due to serious side effects. Amitriptyline poisoning, a condition more dangerous in children than in adults, is one example (Paksu et al., 2014). Given that studies on the therapeutic benefit experienced by juveniles and adolescents and by carriers of low expressing SERT gene variants from TCA treatment have reported mixed results (Hazell et al., 1995; Rajewska-Rager et al., 2008; Perlis et al., 2010; Hazell and Mirzaie, 2013), there is a need to better understand the age- and SERT gene variant-dependency of TCA efficacy as a first step towards developing improved antidepressants for these populations.

TCAs primarily act to block the norepinephrine transporter (NET), but some also block SERT. The ensuing increase in extracellular norepinephrine (NE) and 5-HT is thought to trigger therapeutic downstream events (Frazer, 1997). It has been suggested that a developmental delay in the noradrenergic central nervous system may limit the antidepressant potential of NET targeting TCAs in juveniles and adolescents compared with adults (Bylund and Reed, 2007). However, other reports go against this idea. For example, in rats, innervation of noradrenergic neurons into higher brain regions, such as the cortex, reach adult-like morphology by postnatal day (P) 7 (Loizou, 1972; Lauder and Bloom, 1974; Coyle, 1977; Thomas et al., 1995). Noradrenergic receptors reach adult levels by P14 – P21, and norepinephrine content reaches adult levels by P14 – P42 (Loizou and Salt, 1970; Konkol et al., 1978; Morris et al., 1980; for review see Murrin et al., 2007). In juvenile and adolescent rats, NET expression is reported to be greater or equivalent to that in adults in numerous brain regions (Moll et al., 2000; Sanders et al., 2005). These findings suggest that NET selective TCAs, such as desipramine (DMI), should produce antidepressant-like effects in juvenile (P21) and adolescent (P28) rodents, because the noradrenergic system is relatively established at these ages.

However, there is a paucity of research investigating antidepressant-like effects of TCAs in juvenile and adolescent rodents. In rats, the TCAs imipramine and DMI have been reported to be less effective in producing antidepressant-like effects in the forced swim test (FST) in juveniles than in adults. In contrast, we found that 32 mg/kg DMI produced equivalent antidepressant-like effects in mice aged P21, P28 and P64-90 (young adult) in the tail suspension test (TST) (Mitchell et al., 2013). These behavioral

results were paralleled by [^{3}H]nisoxetine binding in whole hippocampal homogenates, which revealed no difference in NET expression among these ages. While informative, these studies provided no information on how age may affect the potency or maximal effect of DMI to produce antidepressant-like effects in the TST. [^{3}H]Nisoxetine binding assays in hippocampal homogenates also lack the ability to discriminate potential differences in NET expression among hippocampal sub-regions as a function of age, and do not provide information about other brain regions that may be important in mediating antidepressant-like effects.

Determination of the potency and maximal effect of DMI to produce antidepressant-like effects in the TST, as well as establishing the ontogeny of NET expression throughout early postnatal development could help to identify age-dependent mechanisms that may limit the therapeutic benefit of antidepressants. Here, we examined the dose-response relationship for DMI to produce antidepressant-like effects in P21, P28 and P90 SERT-deficient mice, and quantified NET expression in a number of brain regions using autoradiography with [^{3}H]nisoxetine, a NET-selective ligand. SERT-deficient mice were included in this study to assess effects of constitutive reduction in SERT expression, which occurs in humans carrying low expressing gene variants of SERT, on antidepressant-like effects of DMI, and on NET expression and affinity.

MATERIALS AND METHODS

Animals

Naïve male and female SERT wild-type (SERT+/+), heterozygote (SERT+/-) or homozygote knockout (SERT-/-) mice (backcrossed to C57BL/6J for >10 generations) were used for all experiments. Dr. Dennis Murphy (National Institute of Mental Health) provided mice to found the colony. Animals were bred by crossing male and female SERT+/- mice, and SERT-genotypes were identified as previously described (Bengel et al., 1998). Mice were aged P21 (juvenile), P28 (adolescent), and P90-P100 (adult) (Spear, 2000) for all experiments. Animals were housed in a temperature-controlled (24°C) vivarium maintained on a 12/12-hr light/dark cycle (lights on at 7:00 am) in plastic cages (29 cm x 18 cm x 13 cm) containing rodent bedding (Sani-chips, Harlan Teklad, Madison, WI, USA) with free access to food (irradiated rodent sterilizable diet, Harlan Teklad, Madison, WI, USA) and water. Weaning occurred at P28, after which mice were housed with five of their same-sex peers. To avoid possible confounds of treatment effects with litter effects, no more than one mouse from a given litter was assigned to a particular treatment condition. All procedures were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, https://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-use-of-laboratory-animals.pdf), and with the approval of the Institutional Animal Care and Use Committee, The University of Texas Health Science Center at San Antonio.

Tail suspension test

TST experiments were conducted as originally described by Steru et al. (1985) with minor modifications (described in Mitchell et al., 2015). In brief, mice were moved from a housing facility to a testing room then given a 1-2 h acclimation period prior to TST. Experiments were conducted in the afternoon, between 12:00 noon and 5:00 pm. Mice received an intraperitoneal (i.p.) injection of saline 1 h prior to test, followed by an i.p. injection of either DMI (3.2, 10 or 32 mg/kg) or saline vehicle (control condition) 30 min prior to test. This two injection protocol was used to be consistent with previous studies from this laboratory (Baganz et al., 2008; Horton et al., 2013) and with future studies that will examine the effects of drug combinations on immobility time in the TST. Immediately before testing, the distal portion of the tail was secured to a flat aluminum (2 X 0.3 X 10 cm) bar using adhesive tape. A hole on the opposite end of the bar was secured to a hook in a visually isolated white box (40 X 40 X 40 cm). Each mouse was suspended for 6 min while a digital video camera recorded its ventral surface. Immobility was defined as the absence of active movements, and included passive swaying. Immobility time was scored in s by observers blind to the randomly assigned treatment conditions. Mice were tested only once.

Drugs

Desipramine (desmethylimipramine) hydrochloride [Sigma-Aldrich (St. Louis, MO, USA)] was dissolved in physiological saline and injected i.p. at doses expressed as salt weight per kilogram body weight. The injection volume was 10 ml/kg.

Autoradiography

NET density in mouse brain was assessed by quantitative autoradiography using the NET-selective ligand [^{3}H]nisoxetine and methods adapted from Tejani-Butt (1991). Mice were killed by decapitation and brains were flash frozen on powdered dry ice before being stored at -80 °C. Before sectioning, brains were brought to -20 °C in a cryostat (Leica CM 1850, Meyer Instruments, Houston, TX). Coronal sections (20 µm) were collected at the level of plate 12 (prefrontal cortex), plate 47 (hippocampal sub-regions [Cornu Ammonis (CA) 1, 2, 3 and dentate gyrus], amygdala, plate 64 (raphe nuclei), and plate 76 (locus coeruleus) according to Paxinos and Franklin's mouse brain atlas (1997). Sections were thaw mounted onto gelatin-coated microscope slides. Tissue mounted slides were vacuum desiccated for 18-24 h at 4 °C before storing slides at -80 °C. Brain tissue was stored at -80 °C for 2 – 4 weeks, and every experiment contained brain tissue from all groups, including P21, P28 and P90 SERT+/+, SERT+/- and SERT-/- male and female mice. Before incubation, sections on slides were thawed for 1 h in a vacuum desiccator at 4°C to remove excess moisture and maximize brain tissue adherence to slides. The slides were then pre-incubated for 1 h in a 43 mM Tris-HCl, 124 mM NaCl, and 4.3 mM KCl pH 7.4 wash buffer solution at room temperature (~ 24°C) to remove endogenous ligands bound to NET. Incubation was carried out in slide mailers (VWR, USA) filled with 10 ml of 50 mM Tris-HCl, 300 mM NaCl, and 5 mM KCl pH 7.4 ice cold reaction buffer containing [^{3}H]nisoxetine at concentrations of 0.3, 1.0, 3.0, 10, or 3.0 nM for 4 h. Each concentration of [^{3}H]nisoxetine was incubated with 3 brain sections per animal per brain region. Non-specific binding was defined by 2.5 mM mazindol (Pfizer, Groton CT) and was ~9-50 % total binding in low binding regions (i.e. CA1) and ~9-22 % total binding in high binding regions (i.e. locus coeruleus). The

incubation was terminated by three 5 min washes in wash buffer solution at 4 °C, followed by a 5 s dip in de-ionized water at 4 °C. Slides were dried on a slide warmer for 20 min. [^{3}H]Nisoxetine labeled sections were exposed to Carestream Biomax MR film for 6 weeks, along with tritium standards (American Radiolabeled Chemicals, St Louis, MO). Films were developed in a film processor (AFP imaging, Elmsford, NY). A digital imaging system that included a 12-digital bit camera (CFW-1612M, Scion Corp., Frederick, MD), Nikon Lens, Northern Lights Illuminator and Kaiser RS1 copy stand (all from InterFocus Imaging Ltd., Linton, England) was used to capture autoradiogram image. Autoradiograms were calibrated and measured using NIH Image J public access shareware (https://imagej.nih.gov/ij/download.html) on a MacBook (OS 10). Additional brain sections we stained with thionine (FD NeuroTechologies, INC, Columbia, MD, USA) to verify tissue integrity and neuroanatomical brain regions quantified (Figure 2.B and 3.B).

Data analysis

Statistical analyses were performed using Prism 6.0 (GraphPad, San Diego, CA, USA) and NCSS 2007 (Kaysville, UT, USA).

TST:

Dose response curves were generated by administering 3.2, 10, or 32 mg/kg DMI or saline vehicle in juvenile, adolescent or adult mice. Under vehicle conditions, we previously found that time spent immobile varies by age and SERT-genotype (Mitchell et. al., 2013; 2015; 2016). Because such differences were also apparent in the present study, (see vehicle data in Figure 1.A-C), we have expressed data as total immobility in

(s) (Figure 1.A-C), then replotted these data as percent from saline vehicle control (Figure 1.D-F). Within genotype, TST data were analyzed by two-factor ANOVA (age, drug dose) followed by Dunnett's and Tukey's multiple comparisons tests. Sample sizes for TST data included 9 - 14 SERT+/+ males and 10 - 20 SERT+/+ females, 8 - 10 SERT+/- males and 16 - 20 SERT+/- females, and 8 - 10 SERT -/- males and 9 - 14 SERT-/- females, per data point. A multifactor ANOVA showed neither a main effect of sex nor any interaction of sex with other factors ($P = 0.51$ and $P \geq 0.10$, respectively), with the exception of a sex x genotype interaction ($P = 0.008$); however, multiple comparisons failed to show significant sex differences for each of the genotypes. Thus, data for males and females were combined. Maximal effects (Emax) and half-maximally effective dose (ED_{50}) were derived from data in Figure 1.D-F and are summarized in Table 1. Emax was defined as the greatest observed percent change of immobility from saline control. Emax values were analyzed by two-factor ANOVA (age, SERT genotype) followed by Tukey's multiple comparisons test (Table 1). ED_{50} values were calculated using methods detailed in Koek et al. (2009). Briefly, the linear portion of the dose-response curves was analyzed by log-linear regression (effect = slope X log(dose) + intercept) of data from individual subjects. All data are expressed as mean ± standard error of the mean (SEM), except ED_{50} values, which are expressed as the mean. $P < 0.05$ was considered statistically significant.

Quantitative Autoradiography:

[^{3}H]Nisoxetine binding densities were measured from autoradiograms and analyzed using methods described in Mitchell et al., (2016). In brief, non-specific binding was fit to an unweighted linear regression and subtracted from total

[^{3}H]nisoxetine to give specific binding. Unweighted non-linear regression was used to analyze [^{3}H]nisoxetine specific binding data. Saturation binding isotherms were fitted according to a one site model: $Y = Bmax * X / (K_d + X)$ to calculate maximal specific [^{3}H]nisoxetine binding (Bmax) and affinity (K_d) values (Figure 2 and 3). Bmax and K_d values were analyzed with a two-factor (age, genotype) ANOVA (Figure 4 and Table 2). Within genotype- and brain region-matched groups there were no statistically-significant effects of sex ($P \geq 0.09$) or sex and age interactions ($P \geq 0.17$) for [^{3}H]nisoxetine Bmax or K_d values; thus, data from both sexes were combined. Two-factor ANOVA (age, SERT genotype) (Figure 4) followed by Dunnett's *post hoc* test for multiple comparisons was used to analyze mean Bmax and K_d values. All data are expressed as mean ± standard error of the mean (SEM). $P < 0.05$ was considered statistically significant.

Correlations:

As shown in Figure 5.A-E, Pearson's correlation was used to examine the relation between Emax for DMI to reduce immobility time in the TST and maximal specific [^{3}H]nisoxetine binding, as a function of age. Within each brain region, SERT genotype did not significantly influence the relation between Emax for DMI and maximal specific [^{3}H]nisoxetine binding ($P \geq 0.15$; data not shown) so data for SERT+/+, SERT+/- and SERT-/- were best fit with a single line (Figures 5.A-E). All data are expressed as mean ± standard error of the mean (SEM).

RESULTS

The potency of desipramine to reduce immobility time in the tail suspension test depends on age and SERT genotype, while its maximal effect depends on age only.

Clinical studies have shown that therapeutic potential of SSRIs is limited in children (Kirsch et al., 2008). Similar, we have previously found the antidepressant-like response to SSRIs in juvenile mice is limited in comparison to adult mice (Mitchell et al., 2013; Mitchell et al., 2016). As an alternative to SSRIs, we began our study by evaluating the antidepressant-like response to DMI, a potent NET blocker, in SERT+/+ P21, P28 and P90 mice. Using the TST, we found that DMI reduced immobility time in all age groups [$F(3,276) = 35.06$, $P < 0.01$], and younger mice spent overall less time immobile than adult mice [$F(2,276) = 19.73$, $P < 0.01$] (Figure 1.A). An interaction between age and DMI dose showed that younger mice are more sensitive to the anti-immobility effects of DMI than adults [$F(6,276) = 2.34$, $P = 0.03$]. *Post hoc* analysis revealed that immobility time was significantly lower in P21 mice than in P28 and P90 mice after administration of 10 mg/kg DMI ($P < 0.01$), and immobility time was lower in P21 mice than in P90 mice after administration of 3.2 and 32 mg/kg DMI ($P < 0.01$, $P = 0.012$). Likewise, immobility time at 3.2 mg/kg DMI was significantly lower in P28 mice than in P90 mice ($P < 0.01$). 3.2 mg/kg DMI was the lowest effective dose in P21 and P28 mice, and 10 mg/kg was the lowest effective dose in P90 mice ($P < 0.01$). Taken together, the anti-immobility effects of DMI appear to be greater in younger mice than adults.

Clinical studies have also found that the antidepressant effects of SSRIs are limited in individuals with a SERT gene polymorphism that yields a reduction in SERT expression/function (Serretti et al., 2007). We utilized SERT+/- mice, which express 50 percent less SERT than SERT+/+ mice, to evaluate the antidepressant-like potential of NET blockade in individuals with reduced SERT expression/function. DMI reduced immobility time in the TST [$F(3,212) = 16.58$, $P < 0.01$] (Figure 1.B), and younger mice spent overall less time immobile in the TST than adult mice [$F(2,212) = 9.34$, $P < 0.01$]. In contrast with SERT+/+ mice, no interaction was found between age and DMI drug dose in SERT+/- mice [$F(6,212) = 0.81$, $P = 0.57$]. *Post hoc* analysis showed that immobility time in the TST was lower in vehicle-treated P28 mice than vehicle-treated P90 mice ($P < 0.01$). The lowest effective dose for P21 and P90 mice was 3.2 mg/kg DMI ($P < 0.01$). While no dose of DMI significantly reduced immobility time in P28 mice, immobility times following 32 mg/kg DMI trended to be lower than immobility times following vehicle treatment ($P = 0.087$). These data suggest antidepressant-like effects of DMI are not greater in younger SERT+/- mice than in adult mice; however, variation in basal immobility (saline vehicle control) as seen with P28 SERT+/- mice may limit the interpretation of our findings.

While DMI is a potent NET blocker, it does have affinity for SERT. The antidepressant-like effect of DMI in SERT-/- mice was evaluated to investigate the proportion of antidepressant-like response that was SERT-dependent in P21, P28 and P90 mice. Immobility time in the TST was reduced after DMI administration [$F(3,217) = 9.00$, $P < 0.01$], although no dose of DMI significantly lowered immobility time in P28 mice ($P = 0.13$). A main effect of age was also found [$F(2,217) = 6.14$, $P < 0.01$] (Figure

1.C). No interaction between age and drug dose was found [$F(6,217) = 0.29$, $P = 0.94$]. Immobility time was lower in P21 and P90 mice than in P28 mice after administration of 10 mg/kg DMI ($P = 0.015$, $P < 0.01$). The lowest effective dose for P21 and P90 mice was 10 mg/kg DMI ($P < 0.01$; $P = 0.04$). These data suggest that NET blockade is primarily responsible for the antidepressant-like effects of DMI in the TST, with the possible exception of P28 SERT-/- mice where, as for SERT+/- mice, no dose of DMI significantly reduced immobility time (Figure 1.C).

It should be noted that basal immobility time in the TST was lower in SERT-/- mice (Figure 1.C) compared to SERT+/+ mice (Figure 1.A), and P28 SERT+/- mice showed less basal immobility in comparison to P90 SERT+/- mice (Figure 1.B). To account for age and SERT genotype related variations in basal immobility time, data were normalized to a percent of vehicle control (Figure 1.D-F). Analysis of normalized SERT+/+ data was similar to the analysis of raw SERT+/+ data (in s) with minor exceptions. DMI reduced immobility time in all SERT+/+ age groups [$F(3,276) = 36.23$, $P < 0.01$], and a main effect of mouse age was found [$F(2,276) = 10.36$, $P < 0.01$] (Figure 1.D). Younger mice showed greater sensitivity to the anti-immobility effects of DMI in the TST than adult mice [$F(6,276) = 2.58$, $P = 0.02$]. Immobility time was significantly lower in P21 SERT+/+ mice than in P90 mice following 3.2 mg/kg ($P < 0.01$) and 10 mg/kg DMI ($P = 0.04$). Immobility time was lower in SERT+/+ P28 mice than in P90 mice following 3.2 mg/kg DMI ($P < 0.01$). The lowest effective dose for DMI was 3.2 mg/kg in SERT+/+ mice aged P21 and P28 ($P < 0.01$), and the lowest effective dose for P90 mice was 10 mg/kg ($P < 0.01$).

Analysis of normalized SERT+/- data showed that DMI reduced immobility time in the TST [$F(3,212) = 15.7$, $P < 0.01$], with no main effect of age [$F(2,212) = 2.091$, $P = 0.13$] (Figure 1.E). DMI reduced immobility time in all age groups, and did so equally across all age groups [$F(6,212) = 0.46$, $P = 0.84$]. No differences among age groups were found. The lowest effective dose for P21 and P90 SERT+/- mice was 3.2 mg/kg DMI ($P < 0.01$; $P = 0.03$, respectively). The lowest effective dose for P28 SERT+/- mice was 32 mg/kg DMI ($P = 0.02$). By normalizing SERT+/- data, we found that DMI reduced immobility in all age groups, including P28 SERT+/- mice.

Normalized data for SERT-/- mice showed a main effect of DMI [$F(3,217) = 9.01$, $P < 0.01$] and age [$F(2,217) = 4.20$, $P = 0.02$] (Figure 1.F). Like SERT+/- mice, there was no interaction between age and drug dose [$F(6,217) = 0.27$, $P = 0.95$]. After 10 mg/kg DMI, immobility time was significantly lower in P21 and P90 SERT-/- mice than in P28 mice ($P = 0.02$; $P = 0.03$). The lowest effective dose for P21 and P90 SERT-/- mice was 10 mg/kg ($P = 0.02$), and no dose significantly reduced immobility time in P28 mice including 32 mg/kg DMI ($P = 0.17$).

To investigate a possible interaction between postnatal age and SERT genotype on the antidepressant-like response to DMI, we compared the potency (ED_{50}) and maximal effect (Emax) of DMI at different ages and in different genotypes (Table 1). With the exception of P28 SERT+/- and SERT-/- mice, ED_{50} values for DMI were lower in younger mice than in adults. ED_{50} values for DMI varied as a function of age in SERT+/+, SERT+/- and SERT-/- mice [$F(2,205) = 12.57$, $P < 0.01$; $F(2,163) = 3.22$, $P = 0.04$; $F(2,149) = 3.38$, $P = 0.04$; respectively]. In SERT+/+ mice, the ED_{50} value was lower in P21 mice than in P28 mice and P90 mice [$F(1,125) = 6.75$, $P = 0.02$; $F(1,143) =$

25.0, P < 0.01], and P28 mice had a lower ED_{50} value than P90 mice [F(1,143) = 5.2, P = 0.02]. In SERT+/- mice ED_{50} values were lower in P21 mice than in P28 mice [F(1,105) = 5.7, P = 0.02]. In SERT-/- mice ED_{50} values were lower in P21 mice than in P28 mice [F(1,96) = 9.33, P < 0.01]. Among SERT genotype comparisons showed an effect of SERT genotype in P28 mice [F(2,167) = 5.50, P < 0.01]. P28 SERT+/+ mice showed a lower ED_{50} than P28 SERT-/- mice [F(1,116) = 12.25, P < 0.01]. SERT- genotype had no significant main effect on ED_{50} values in P21 or P90 mice [F(2,177) = 1.29, P = 0.28; F(2,193) = 0.82, P = 0.44]. In P28 mice, an effect of SERT genotype was found [F(2,167) = 5.50, P < 0.01]. P28 SERT+/+ mice had a lower ED_{50} value than P28 SERT-/- mice [F(1,116) = 12.25, P < 0.01].

The Emax for DMI to reduce immobility time in the TST was significantly greater in young mice than in adults [F(2,178) = 7.06, P < 0.01]. P21 SERT+/+ mice showed a significantly greater Emax than P90 SERT+/+ mice (P = 0.046). As well, the Emax for DMI in P21 SERT-/- mice was greater than P28 SERT-/- mice (P = 0.02). SERT genotype did not affect the Emax for DMI to reduce immobility time [(2,178) = 1.043, P = 0.35], and SERT genotype did not interact with the effects of age. [F(4,178) = 0.94, P = 0.44].

Collectively, the ED_{50} values for DMI to decrease immobility time in the TST were lowest in P21 mice, regardless of SERT genotype. Emax values for DMI were greater in P21 mice than in P28 and P90 mice. The role of SERT genotype in antidepressant-like response to DMI was most apparent in P28 mice, where drug potency decreased with the reduction and loss of SERT, although a similar trend was observed with P21 mice as well (Table 1).

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NET expression increased with age in some noradrenergic terminal regions and decreased with age in the locus coeruleus.

In an effort to explain the age-dependent antidepressant-like response to DMI, NET binding densities were quantified in limbic (CA1, CA2, CA3, dentate gyrus, prefrontal cortex and amygdala) and cell body (dorsal raphe and locus coeruleus) regions using quantitative autoradiography to measure specific [^{3}H]nisoxetine binding. Figures 2.A and 3.A show representative autoradiograms. Figures 2.B and 3.B are thionine stained tissue sections to confirm tissue integrity and to highlight brain regions quantified where significant differences were found. These sections are a representative enlargement of the boxed areas shown in the top right panels of Figures 2.A and 3.A. Figures 2.C and 3.C show summary data for specific binding in SERT+/+ mice in the CA3 region of hippocampus and locus coeruleus, respectively. Bmax and Kd values were analyzed with a two-factor (age, genotype) ANOVA (Figure 4 and Table 2) to determine how age and SERT-genotype influence NET binding densities in brain regions of potential importance for TCAs antidepressant-like response. These results are described below.

[^{3}H]Nisoxetine Bmax and K_d values in terminal regions:

In the hippocampus, specific [^{3}H]nisoxetine Bmax values significantly increased with age in the CA1 and CA3 [$F(2,52) = 4.53$, $P = 0.02$; $F(2,52) = 5.40$, $P < 0.01$], but not in the CA2 region of hippocampus or dentate gyrus [$F(2,52) = 2.71$, $P = 0.08$; $F(2,52) = 0.42$, $P = 0.66$] (Figure 4.A-D). Bmax values in P90 SERT+/+ mice were greater than in P21 SERT+/+ mice in CA1 and CA3 ($P = 0.02$; $P < 0.01$) (Figure 4.A,C,). Bmax values did not vary as a function of SERT genotype in the CA1, CA2, CA3

regions of hippocampus or dentate gyrus [F(4,52) = 1.60, P = 0.19; F(2,52) = 0.21, P = 0.80; F(2,52) = 0.78, P = 0.46; F(2,52) = 0.87, P = 0.42, respectively]. Additionally, no interactions between age and SERT genotype on Bmax values were found in these regions [F(2,52) = 1.76, P = 0.18; F(4,52) = 0.57, P = 0.69; F(4,52) = 0.68, P = 0.61; F(2,52) = 0.42, P = 0.66, respectively].

While not statistically significant, Bmax values tended to increase with age in the prefrontal cortex and amygdala [F(2,52) = 3.04, P = 0.056; F(2,52) = 2.32, P = 0.11] (Table 2). Among age comparisons showed greater Bmax values for P90 SERT+/+ mice than P21 SERT+/+ mice in the prefrontal cortex (P = 0.05), and Bmax values were greater in P90 SERT+/+ mice than P28 SERT+/+ mice in the amygdala (P < 0.01). As for hippocampus, Bmax values in the prefrontal cortex and amygdala did not vary as a function of SERT genotype [F(2,52) = 1.30, P = 0.28; F(2,52) = 1.81, P = 0.18, respectively]. No interaction between age and SERT genotype on Bmax values was found in these regions [frontal cortex: F(4,52) = 0.79, P = 0.54; amygdala: F(4,52) = 1.30, P = 0.28]. Within the dorsal raphe, Bmax values were not dependent on either age or SERT genotype [F(2,52) = 0.78, P = 0.46; F(2,52) = 0.36, P = 0.70] (Table 2). No interaction between age and SERT genotype on Bmax values was found [F(4,52) = 0.58, P = 0.68].

K_d values in all terminal regions ranged from 1.0 – 5.5 nM and were not dependent on age (P ≥ 0.44) or SERT genotype (P ≥ 0.5).

[^{3}H]Nisoxetine Bmax and K_d values in cell body regions:

Specific [^{3}H]nisoxetine Bmax values decreased with age in the locus coeruleus [$F(2,52) = 5.11$, $P < 0.05$] (Figure 4.E): Bmax values were greater in P21 SERT-/- mice than in P90 SERT-/- mice ($P = 0.03$). Bmax values did not appear to depend on SERT genotype [$F(2,52) = 2.53$, $P = 0.09$] and no interaction between age and SERT genotype was found [$F(4,52) = 1.00$, $P = 0.41$].

In the locus coeruleus K_d values ranged from 0.52 – 0.75 nM and did not depend on age ($P = 0.67$) or SERT genotype ($P = 0.25$).

Relationship between maximal antidepressant-like effects of DMI in the tail suspension test and maximal binding values for [^{3}H]nisoxetine in hippocampus and locus coeruleus.

Terminal regions:

Figure 5. A-D shows a negative relation between Emax values for DMI's anti-immobility effects in the TST and Bmax values for specific [^{3}H]nisoxetine binding in CA1, CA2 and CA3 regions and dentate gyrus of hippocampus of P21, P28 and P90, SERT+/+, SERT+/- and SERT-/- mice. In all regions, the relation between Emax and Bmax was not significantly influenced by SERT genotype ($P \geq 0.15$); thus, the data were pooled across genotype and were fitted with a single line (Pearson's correlation: $r = -0.66$, $r = -0.74$, $r = -0.65$, $r = -0.26$; for CA1, CA2, CA3 and dentate gyrus).

Cell body regions:

Figure 5.E shows a positive relation between Emax values for DMI's anti-immobility effects in the TST and Bmax values for specific [^{3}H]nisoxetine binding in locus coeruleus. As for hippocampus, the relation between Emax and Bmax in the

locus coeruleus was not affected by SERT genotype ($P = 0.88$); data from SERT+/+, SERT+/- and SERT-/- mice were therefore pooled and fitted with a single line (Pearson's correlation: $r = 0.41$).

DISCUSSION

Here, we found the potency and Emax of DMI to be greater in juvenile (P21) and adolescent (P28) SERT+/+ mice than in adult (P90) SERT+/+ mice (Table 1). Regardless of SERT genotype, NET expression, quantified by specific [^{3}H]nisoxetine binding using autoradiography, increased with age in hippocampus (Figure 4.A-D), and decreased with age in locus coeruleus (Figure 4.E). Age-related changes in Emax for DMI to reduce immobility were negatively related with Bmax values for specific [^{3}H]nisoxetine binding in hippocampus and positively related with Bmax values in locus coeruleus (Figure 5.A-E). Thus, there is an inverse relation between maximal antidepressant-like response following DMI and NET expression in hippocampus, where antidepressant-like response to DMI decreases with age and NET expression increases. In contrast, NET expression in the locus coeruleus and antidepressant-like response to DMI decreases with increasing age. These data reveal a complex relationship between antidepressant-like response to DMI and NET expression, suggesting that the antidepressant-like effect of DMI may be reliant on brain regions such as the locus coeruleus.

The TCA imipramine, a NET and SERT blocker, is known to reduce immobility time of adolescent mice (P28 and P35) in the FST, an assay of antidepressant-like response (Bourin et al., 1998; David et al., 2001; Mason et al., 2009). Adolescent (P28 and P35) mice also respond to the anti-immobility effects of imipramine and DMI in the TST (Mason et al., 2009; Mitchell et al., 2013), and DMI reduces immobility time in the FST in adolescent (P28 and P30) rats (Pechnick et al., 2008; Reed et al., 2008). Our SERT+/+ mouse data agree with literature showing that adolescents (P28) are sensitive

to the antidepressant-like effects of DMI (Mitchell et al., 2013). We found that juvenile (P21) SERT+/+ mice are more sensitive to the antidepressant-like effects of DMI in the TST than P28 mice. These data deviate from experiments using juvenile (P21) rats showing that DMI does not significantly reduce FST immobility (Reed et al., 2008). Possible reasons for this discrepancy are a difference in species (mice vs rats), in behavioral assay (TST vs FST) and in injection schedule. The FST and TST differ in their sensitivity to detect antidepressant-like activity, which can vary with rodent strain, thus discrepancies such as this are expected (Cryan et al., 2005).

The SERT-deficient mouse provides a model of SERT gene variants that confer a reduction in SERT expression and/or function and a reduction in SSRI efficacy (Fox et al., 2007; Serretti et al., 2007; Homberg and Lesch, 2011). Our findings are consistent with reports that DMI and imipramine reduce immobility in adult SERT+/+, SERT+/- and SERT-/- mice in the TST (Figure 1; Holmes et al., 2002). Studies investigating treatment outcomes of patients with low expressing SERT gene variants treated with TCAs are limited. Reports using duloxetine, a SERT and NET blocker, and nortriptyline, a NET selective blocker, have failed to show any association with treatment outcome and low expressing SERT gene variants (Rajewska-Rager et al., 2008; Perlis et al., 2010). Our results from P28 SERT+/- mice suggest that DMI may have especially limited therapeutic efficacy in adolescents harboring low expressing SERT gene variants, although the exact mechanism for this remains unknown.

ED_{50} values increased with age from P21, confirming a similar effect of age in adult (P90) to middle-aged (P300) mice (Table 1; Mitchell et al., 2015). Like DMI potency, Emax values were greatest in SERT+/+ P21 and P28 mice compared with

adults (Table 1). These findings are consistent with a study showing a greater antidepressant-like Emax of imipramine in adolescent mice (P28) than in adults using the FST (David et al., 2001). Emax values were not dependent on SERT genotype, which is consistent with studies showing no difference in Emax values between P90 SERT+/+ and P90 SERT+/- or P90 SERT-/- mice (Mitchell et al., 2015).

It should be noted that we cannot rule out other age-dependent factors as modulators of antidepressant-like response, i.e., expression and function of noradrenergic receptors, pharmacokinetic parameters, and expression of NE synthesizing enzymes (Murrin et al., 2007). Future studies are needed to better understand the ontogeny of the noradrenergic system and how changes in these parameters may influence the response to psychoactive therapeutics.

The therapeutic benefit of a drug may be impacted by its tolerability. Pediatric patients treated with TCAs consistently showed side effects such as vertigo, tremors, low blood pressure and dry mouth (Hazell and Mirzaie, 2013). Side effects do not necessarily limit antidepressant-like drug effects in the TST. Thus, our findings suggest TCAs may be efficacious antidepressants in juveniles (lower ED_{50} and greater Emax), but low tolerability of TCAs may limit the therapeutic benefit of TCAs in pediatric patients. Therefore, our results encourage further investigation of the utility of NET, and/or dual NET and SERT blockers in the treatment of pediatric depression and the development of new drugs that target transporters for NE and 5-HT but have fewer side effects.

To elucidate the mechanism underlying age-related changes in the antidepressant-like response to DMI we used quantitative autoradiography to assess

[^{3}H]nisoxetine binding to NET (Figure 2 and 3). Specific [^{3}H]nisoxetine binding densities generally increased with age in noradrenergic terminal regions. Bmax values were greater in adult CA1 and CA3 hippocampal regions than in younger mice (Figure 4.A,C). Bmax values trended to increase with age in the CA2, dentate gyrus, prefrontal cortex and amygdala (Figure 4.B,D and Table 1). These findings partially diverge from experiments using [^{3}H]nisoxetine saturation binding with whole hippocampus mouse homogenate, where no age related differences in Bmax values among P21, P28 and P90 mice were found (Mitchell et al., 2013). It is conceivable that hippocampal sub-region differences in NET expression, which can be revealed by quantitative autoradiography, limited the ability to detect age related changes in Bmax values for [^{3}H]nisoxetine binding when using hippocampal homogenate preparations. Notably, the K_d values for [^{3}H]nisoxetine here (0.5 – 5.5 nM) are consistent with K_d values from hippocampal homogenate preparations (2.5 – 7.4 nM) (Mitchell et al., 2013).

In rat hippocampus, triphasic [^{3}H]nisoxetine binding patterns from birth through adulthood have been observed (Sanders et al., 2005). Binding densities increased postnatally, peaked between P15 – P25 and decreased into adulthood. In contrast, we found that Bmax values for [^{3}H]nisoxetine binding increased with age from P21 to P90 (CA1 and CA3) or remain unchanged (CA2 and dentate gyrus) (Figure 4.A-D). In the locus coeruleus of rats, [^{3}H]nisoxetine binding densities were found to peak at P10 then decrease into adulthood (Sanders et al., 2005). Our data in mice locus coeruleus are consistent with this finding (Figure 4.E).

It is important to evaluate NET expression in SERT-deficient mice because non-SERT transporters of 5-HT, such as NET, could upregulate to compensate for the loss

of SERT (Daws et al., 1998; 2005; 2009; Baganz et al., 2008). We found that Bmax for [^{3}H]nisoxetine binding was similar among SERT genotypes (Figure 4), which is consistent with previous findings showing that NET expression remains unchanged in the CA3 region of hippocampus (Montañez et al., 2003). NET protein levels appear to be uninfluenced by a constitutive reduction or loss of SERT expression.

Increased NET expression in hippocampus as a function of increasing age correlated with a reduction in maximal antidepressant-like effect of DMI (Figure 5.A-D). This relationship was reversed in the locus coeruleus such that decreasing NET expression as a function of age correlated with a reduction in maximal antidepressant-like effect for DMI (Figure 5.E). The antidepressant action of TCAs is hypothesized to occur by increasing extracellular levels of NE, primarily via NET blockade in noradrenergic terminal regions, such as the hippocampus (Przegaliński et al., 1997; Herr et al., 2012). Given that we found an inverse relationship between NET expression in hippocampus and antidepressant-response (increasing and decreasing, respectively) as a function of age, our data suggest brain regions other than hippocampus, such as the locus coeruleus, are likely prominent contributors to the antidepressant-like effects of DMI in the TST and warrant study.

This study evaluated the dose-dependency of the antidepressant-like effects of DMI and provided a survey of [^{3}H]nisoxetine binding to brain NET in juvenile, adolescent and adult SERT wild-type and SERT-deficient mice. Regardless of SERT genotype, DMI potency and Emax were generally greater in juvenile mice than in adults. Of note, P28 SERT+/- and -/- were relatively insensitive to the antidepressant-like effects of DMI, suggesting that NET blocking antidepressants may be especially

ineffective in adolescents harboring low expressing/function variants of SERT, whereas the opposite may be true for juveniles (of any SERT genotype). Regardless of SERT genotype, NET binding increased with age in several noradrenergic terminal regions and decreased with age in the locus coeruleus. These findings help lay the ground-work for future studies investigating the age- and SERT genotype-dependency of NET acting drugs, such as reboxetine. Studies to examine the mechanism by which age-dependent variation in NET expression contribute to antidepressant response of NET blockers remains an important avenue for future inquiries. The ultimate goal is to develop effective antidepressants for patients whose symptoms of depression are resistant to treatment with SSRIs.

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AUTHORS CONTRIBUTIONS

Participated in research design: Mitchell NC, Gould GG, Koek W, and Daws LC.

Conducted experiments: Mitchell NC, Bowman MA and Gould GG.

Performed data analysis: Mitchell NC.

Wrote or contributed to the writing of the manuscript: Mitchell NC, Bowman MA, Gould GG, Koek W, and Daws LC.

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FOOTNOTES

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a) Data from this manuscript are reprinted in a thesis titled “Mechanisms contributing to lack of antidepressant efficacy in juveniles and adolescents: Discovering novel targets for improved therapeutics” (Publication date 6/2016).

b) Reprint request for thesis will be by Nathan Mitchell, 7703 Floyd Curl Drive, San Antonio, TX, 78229-3900, USA.

FIGURE LEGENDS

Figure 1. Influence of age and SERT genotype on the antidepressant-like effect of DMI. (A) Dose-dependent reductions in immobility time in the TST in P21, P28 and P90 SERT+/+ mice (B) SERT+/- mice and (C) SERT-/- mice. (D) Data from figure 1.A expressed as a percent of vehicle control. (E) Data from figure 1.B expressed as a percent of vehicle control. (F) Data from figure 1.C expressed as a percent of vehicle control. Data obtained in males and females are pooled, because a multifactor ANOVA (sex, genotype, DMI) showed no main effect or interaction of sex with other factors ($P > 0.05$) with one exception between sex and genotype ($P < 0.01$); however, multiple comparisons failed to show significant sex differences for each genotype ($P > 0.05$). Data are mean ± SEM. Filled symbols represent data points that are significantly different from SERT genotype- and age-matched vehicle control as determined by Dunnett's *post hoc* multiple comparisons test following a two-factor ANOVA. * $P < 0.05$, * * $P < 0.01$ significant difference from SERT genotype matched P90 and $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$ significant difference from SERT genotype matched P28 with Tukey's post hoc multiple comparisons test after a two-factor ANOVA. SERT+/+ n = 21-31 (males n = 9-14 and females n = 10-20, pooled); SERT+/- n = 16-20 (males n = 8-10 and females n = 8-12, pooled); SERT-/- n = 18-23 (males n = 8-10 and females n = 9-14, pooled), per data point.

Figure 2. Specific [^{3}H]nisoxetine binding to NET in hippocampal regions as a function of age and SERT genotype. Brain sections from P21, P28 and P90 SERT deficient mice incubated with the NET-specific ligand [^{3}H]nisoxetine. Non-specific binding was defined by mazindol (2.5 mM). (A) Representative coronal sections at the

level of plate 47 (Paxinos and Franklin, 1997) in SERT+/+, SERT+/- and SERT-/- mice age P21, P28 or P90. Boxed area in (A) is enlarged in (B), which shows representative thionine stained brain sections labeled with hippocampal regions quantified, which include the CA1, CA2, CA3 and dentate gyrus (DG). (C) Example of saturation binding isotherms used to calculate Bmax and K_d values. Curves include specific [^{3}H]nisoxetine binding values for the CA3 of P21, P28 and P90 SERT+/+ mice. There was no main effect of sex on Bmax or K_d values, so male and female data are pooled ($P > 0.05$). Bmax values are summarized in Figure 4. There were no significant differences in K_d among ages or between SERT+/+ and SERT+/- mice. For SERT+/+ n = 5-9 (males n = 4 and females n = 3-5, pooled), SERT+/- n = 6-10 (males n = 3-5 and females n = 2-4, pooled) and SERT-/- n = 4-7 (males n = 2-4 and females n = 2-4, pooled), mice per group. See Table 2 and Figure 4 for summary of data.

Figure 3. Specific [^{3}H]nisoxetine binding to NET in locus coeruleus as a function of age and SERT genotype. Brain sections from P21, P28 and P90 mice were incubated with increasing concentrations of [^{3}H]nisoxetine. Non-specific binding was defined by mazindol (2.5µM). (A) Representative coronal sections at the level of plate 76 (Paxinos and Franklin, 1997) in SERT+/+, SERT+/- and SERT-/- mice age P21, P28 or P90. Boxed area in (A) is enlarged in (B), which shows a representative thionine stained brain sections including locus coeruleus (LC). (C) Example of saturation binding isotherms used to calculate Bmax and K_d values. Curves include specific [^{3}H]nisoxetine binding values for the LC of P21, P28 and P90 SERT+/+ mice. Bmax values for this region are summarized in Figure 4. There was no main effect of sex on Bmax or K_d values, so male and female data are pooled ($P > 0.05$). For SERT+/+ n =

5-9 (males n = 4 and females n = 3-5, pooled), SERT+/- n = 6-10 (males n = 3-5 and females n = 2-4, pooled) and SERT-/- n = 4-7 (males n = 2-4 and females n = 2-4, pooled), mice per group. See Table 2 and Figure 4 for summary of data.

Figure 4. Bmax for [^{3}H]nisoxetine binding to SERT in SERT+/+ and SERT+/- mice aged P21, P28 and P90. Bmax values from P21, P28 and P90 SERT+/+, SERT+/- and SERT-/- mice in the CA1 (A), CA2 (B), CA3 (C), dentate gyrus (D) and locus coeruleus (E) were determined from 1-site curve fits as described in methods section. Data are mean ± SEM. * $P < 0.05$ represent significant difference from SERT genotype-matched P90, Dunnett's *post hoc* multiple comparisons test after a two-factor ANOVA (age, SERT genotype). Data are mean ± SEM pooled from male and female mice. For SERT+/+ n = 5-9 (males n = 4 and females n = 3-5, pooled), SERT+/- n = 6-10 (males n = 3-5 and females, n = 2-4 pooled) and SERT-/- n = 4-7 (males n = 2-4 and females n = 2-4, pooled), mice per group.

Figure 5. Relationship between Emax values for DMI to produce antidepressant-like effects in the TST and Bmax values for specific [^{3}H]nisoxtine binding in locus coeruleus as a function of age and SERT genotype The CA1 (A), CA2 (B), CA3 (C), dentate gyrus (D) and locus coeruleus (E) are shown. Relationship between Emax and Bmax did not vary by SERT genotype, thus one line was used to fit data regardless of genotype. Data taken from Figure 1.D-F (SERT+/+: n = 21-31, SERT+/-: n = 16-20, SERT-/-: n = 18-23, per data point) and Figure 4.A-E (SERT+/+: n = 5-9, SERT+/-: n = 6-10, SERT-/-: n = 4-7, per age group). Data are mean ± SEM, male and female data are pooled.

TABLES

Table 1. Effect of age and SERT genotype on the potency (ED_{50}) and maximal effect (Emax) of DMI to reduce immobility time in the TST.

Age	SERT	ED_{50} (mg/kg)	Emax (%Δ control)
P21	+/+	7.5 *, #	70.1 ± 5.4 *
	+/-	17.4 #	68.5 ± 7.0
	-/-	17.1 #	62.4 ± 8.9 #
P28	+/+	23.7 *	57.5 ± 10.6
	+/-	36.3	41.3 ± 8.6
	-/-	45.2 †	32.3 ± 9.2
P90	+/+	37.3	47.0 ± 6.1
	+/-	29.1	47.3 ± 7.7
	-/-	30.5	51.9 ± 9.2

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Table 1. Influence of age and SERT genotype on the potency (ED_{50}) and maximal effect (Emax) of DMI to reduce immobility time in the TST. Data are mean ± SEM; values calculated from data shown in figures 1.D, E and F; * $P < 0.05$ difference from SERT genotype matched P90; $^{\#}$ $P < 0.05$ difference from SERT genotype matched P28; † $P < 0.05$ difference from age matched SERT+/+ group; for ED_{50} comparisons made by F ratio test to compare intercepts; for Emax comparisons made by Tukey's post hoc multiple comparisons test after a two-factor ANOVA; because there were no statistically significant sex differences, data for male and female mice were pooled; SERT+/+ n = 21-31 (males n = 9-14 and females n = 10-20, pooled); SERT+/- n = 16-20 (males n = 8-10 and females n = 8-12, pooled); SERT-/- n = 18-23 (males n = 8-10 and females n = 9-14, pooled), per data point.

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Table 2. Summary of Bmax values for specific [^{3}H]nisoxetine binding in SERT+/+, SERT+/- and SERT-/- mice.

Genotype	P21	P28	P90
Prefrontal Cortex			
SERT+/+	228 ± 23*	275 ± 26	314 ± 36
SERT+/-	229 ± 23	251 ± 21	290 ± 30
SERT-/-	206 ± 19	271 ± 35	225 ± 26
Amygdala			
SERT+/+	193 ± 27	137 ± 14*	233 ± 29
SERT+/-	150 ± 29	165 ± 14	191 ± 24
SERT-/-	157 ± 24	146 ± 20	155 ± 21
Dorsal Raphe			
SERT+/+	619 ± 40	551 ± 35	539 ± 35
SERT+/-	560 ± 57	562 ± 31	578 ± 27
SERT-/-	565 ± 20	543 ± 24	536 ± 36

Table 2. Summary of Bmax values for specific [^{3}H]nisoxetine binding in SERT+/+, SERT+/- and SERT-/- mice. Data are mean ± SEM fmol/mg pr. * P < 0.05 different from SERT genotype matched P90 group; Dunnett's multiple comparisons test after two-factor ANOVA. For SERT+/+ n = 5-9 (males n = 4 and females n = 3-5, pooled), SERT+/- n = 6-10 (males n = 3-5 and females n = 2-4, pooled) and SERT-/- n = 4-7 (males n = 2-4 and females n = 2-4, pooled), mice per group.

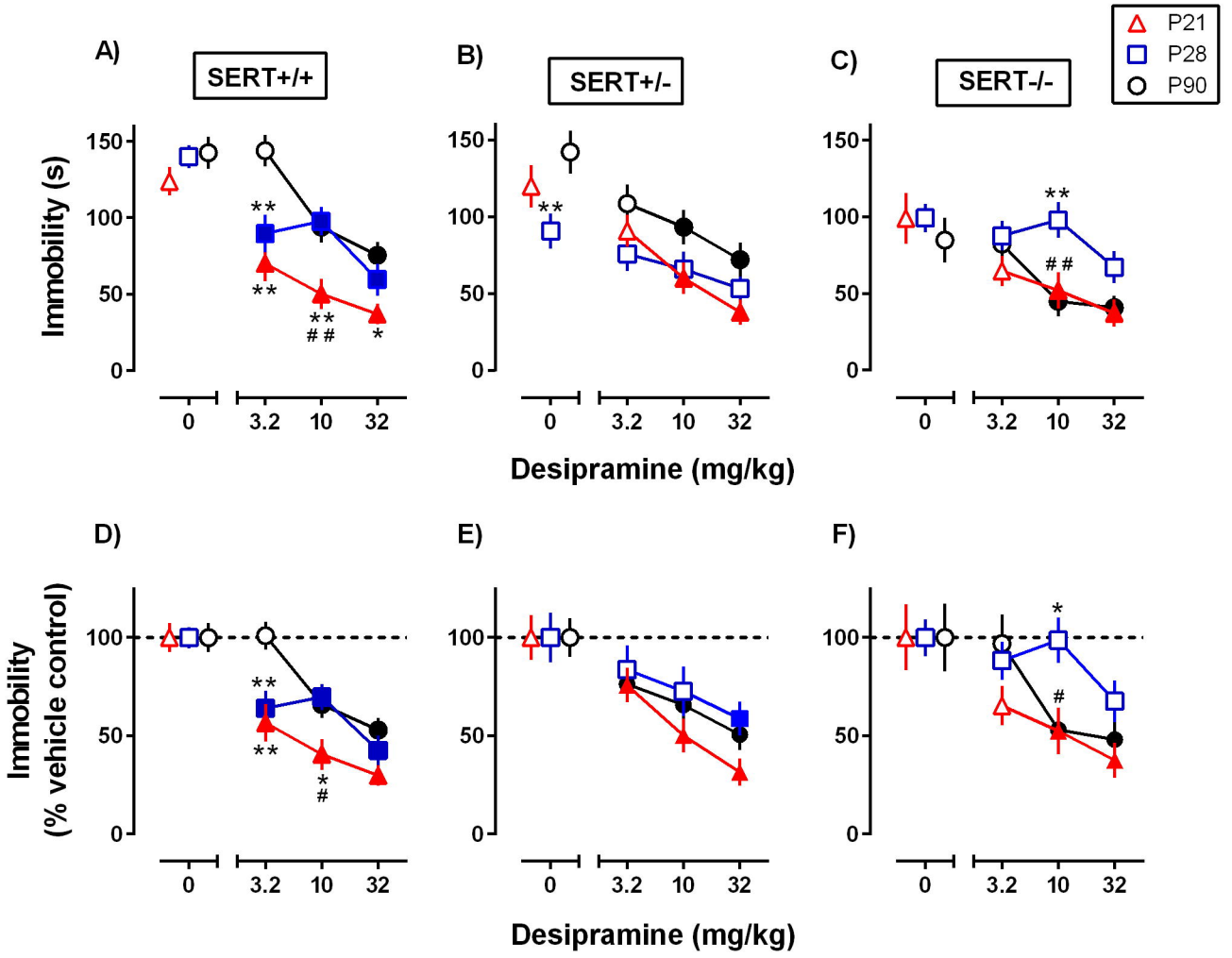
A)
SERT+/+
B)
SERT+/-
C)
SERT-/-
P21
P28
P90
Immobility (s)
150
100
50
0
0
3.2
10
32
Desipramine (mg/kg)
D)
E)
F)
Immobility
(% vehicle control)
100
50
0
Desipramine (mg/kg)

Figure 1.

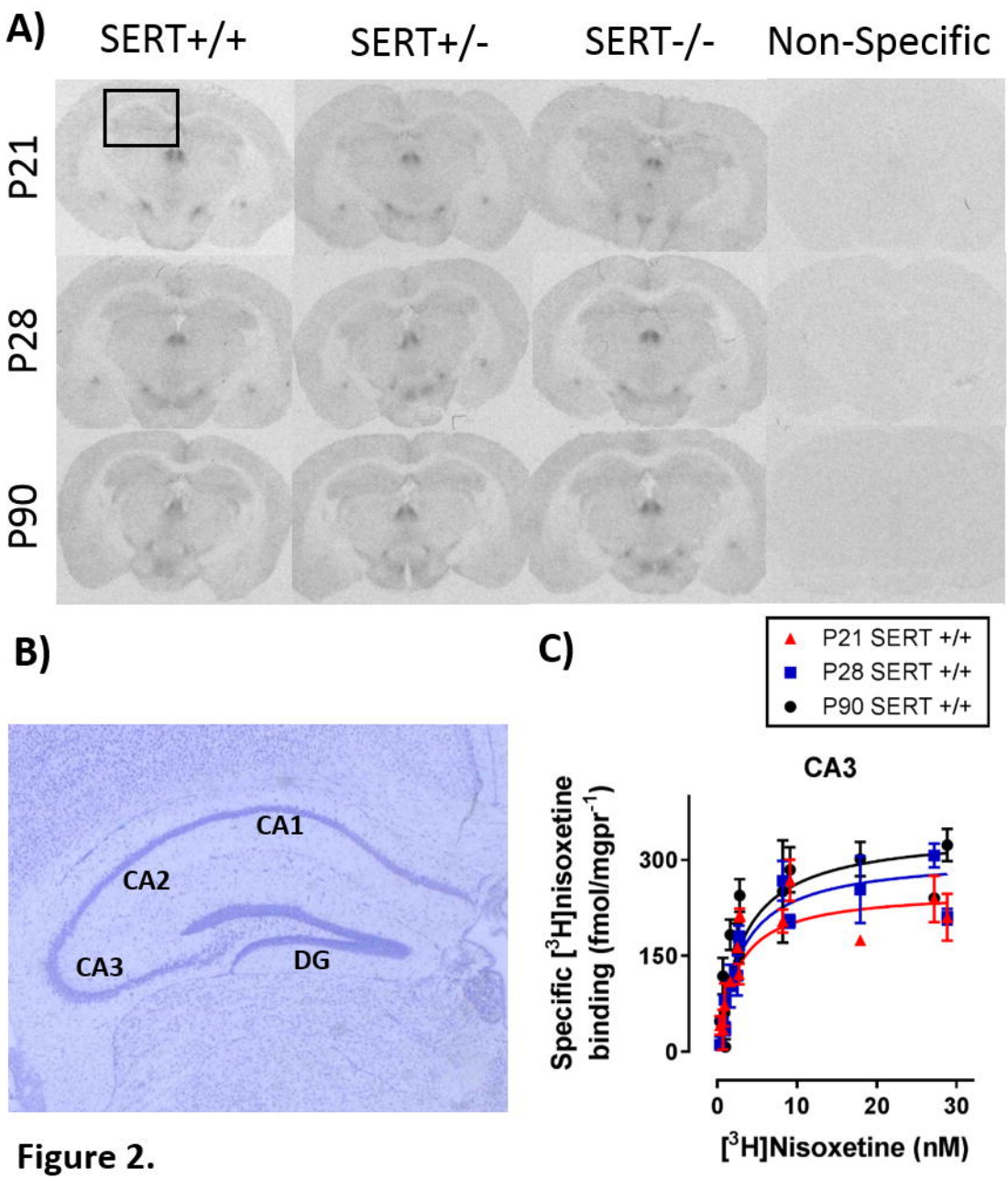
A)
SERT+/+
SERT+/-
SERT-/-
Non-Specific
P21
P28
P90
B)
CA1
CA2
CA3
DG
C)
P21 SERT +/+
P28 SERT +/+
P90 SERT +/+
CA3
Specific [3H]nisoxetine binding (fmol/mgpr-1)
300
150
0
0
10
20
30
[3H]Nisoxetine (nM)

Figure 2.

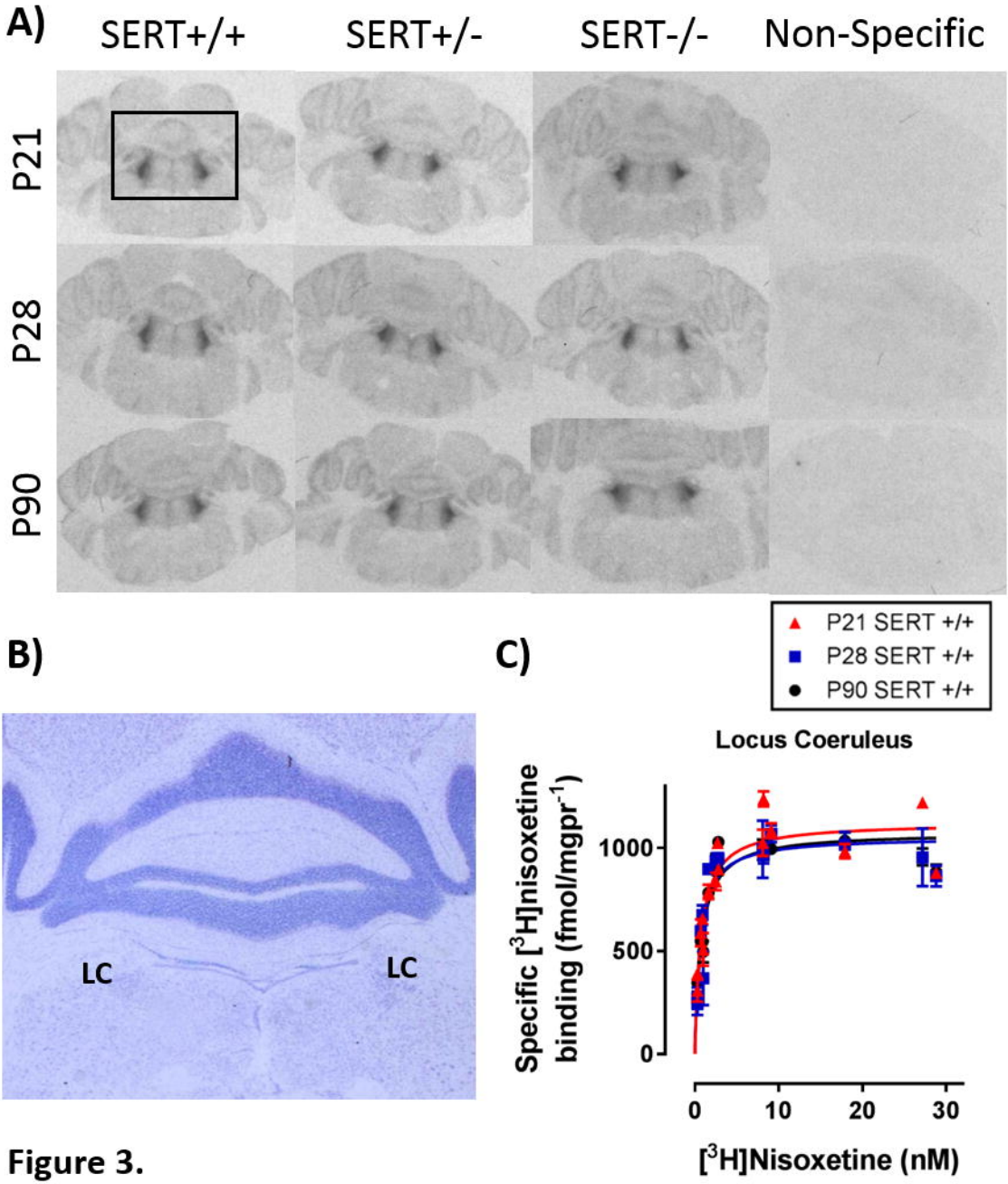
A)
SERT+/+
SERT+/-
SERT-/-
Non-Specific
P21
P28
P90
B)
LC
LC
C)
P21 SERT +/+
P28 SERT +/+
P90 SERT +/+
Locus Coeruleus
Specific [3H]nisoxetine binding (fmol/mgpr-1)
1000
500
0
0
10
20
30
[3H]Nisoxetine (nM)

Figure 3.

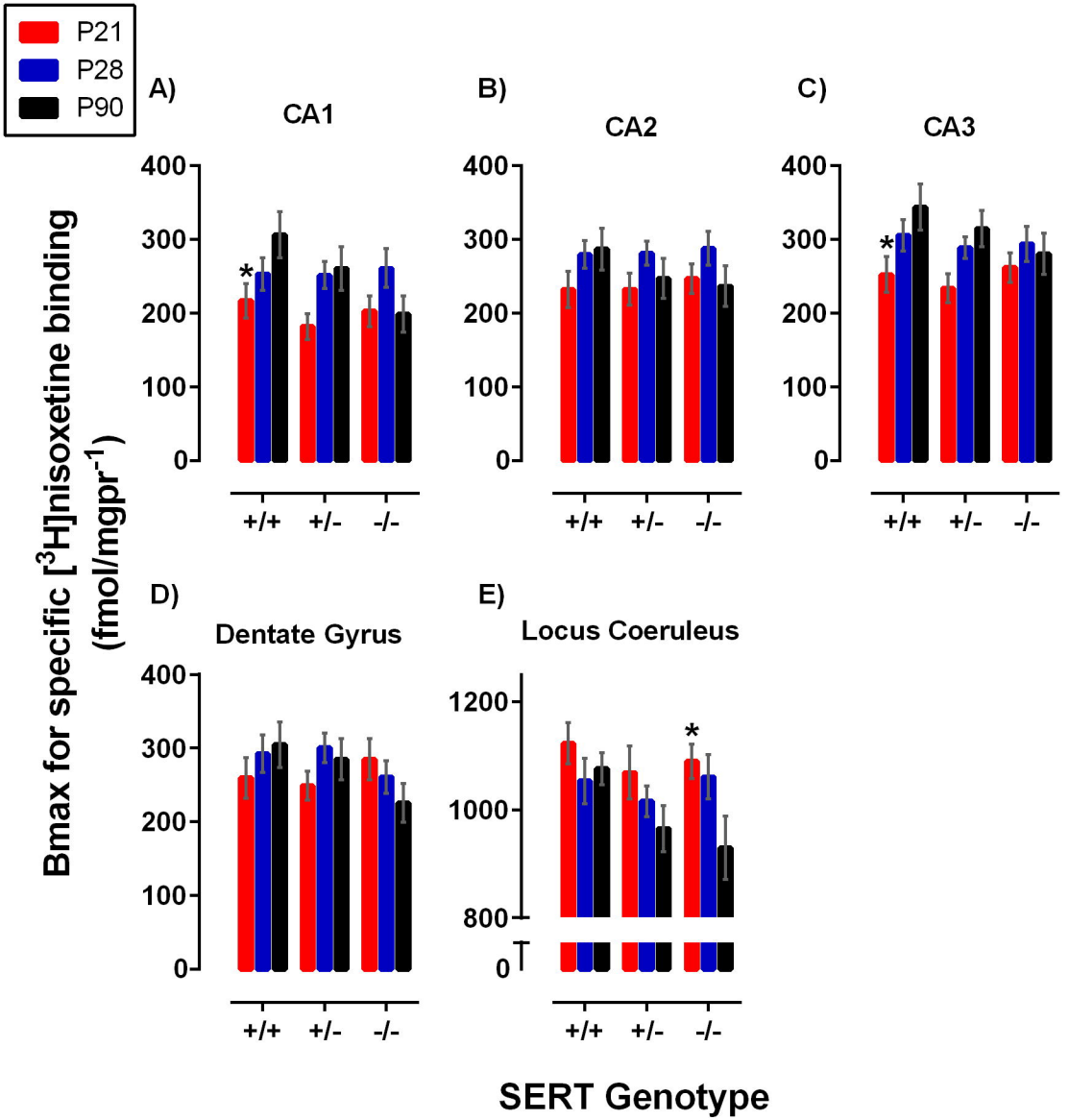
P21
P28
P90
A)
CA1
B)
CA2
C)
CA3
D)
Dentate Gyrus
E)
Locus Coeruleus
Bmax for specific [3H]nisoxetine binding (fmol/mgpr-1)
400
300
200
100
0
1200
1000
800
+/+
+/-
-/-
*
SERT Genotype

Figure 4.

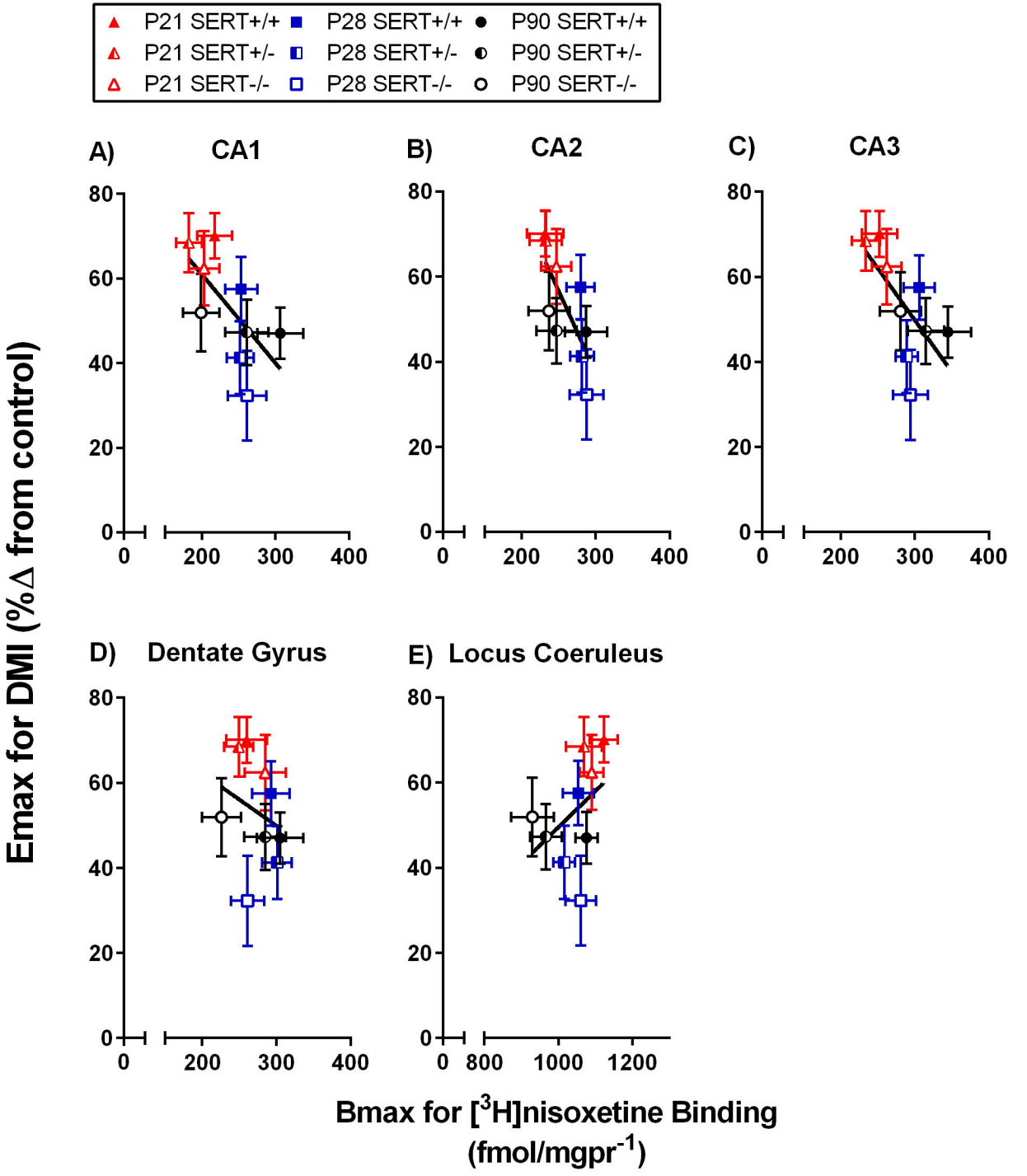
P21 SERT+/+
P28 SERT+/+
P90 SERT+/+
P21 SERT+/-
P28 SERT+/-
P90 SERT+/-
P21 SERT-/-
P28 SERT-/-
P90 SERT-/-
A) CA1
B) CA2
C) CA3
D) Dentate Gyrus
E) Locus Coeruleus
Emax for DMI (%Δ from control)
Bmax for [3H]nisoxetine Binding (fmol/mgpr-1)

Figure 5.